Appendix E

Variant Selection for Production of Drug Metabolites and Diversified Lead Compounds

Table E.1: Identity of engineered P450 BM3 variant panel: enzyme family, name, sequence, number of mutations from closest wild-type parent, and required oxidant

Entry	Family	Name[a]	Sequence	Mutations vs. WT	Selection Criteria	Preferred Oxidant
1	WT	P450 BM3	WT	0	fatty acid	O_2
2	WT	CYP102A2	CYP102A2	0	fatty acid	O_2
3	WT	CYP102A3	CYP102A3	0	fatty acid	O_2
4	WT	A1	CYP102A1 Heme Domain Only	0	none	H_2O_2
5	WT	A2	CYP102A2 Heme Domain Only	0	none	H_2O_2
6	WT	WT F87A	WT F87A	1	none	O_2
7	Propranolol	9C1	**21B3** I102T A145V L324I I366V E442K	14	Propranolol	O_2
8	Propranolol	D6H10	**9C1** L75H V78E A82P	17	Propranolol	O_2
9	Propranolol	DE10	**9C1** A74V A82L A87G	16	Propranolol	H_2O_2
10	Propranolol	2C11	**DE10** K24R R47H	18	Propranolol	H_2O_2
11	Chimera	11113311	chimera	35	none	H_2O_2
12	Chimera	12112333	chimera	96	none	H_2O_2
13	Chimera	21112233	chimera	98	none	H_2O_2
14	Chimera	21112331	chimera	85	none	H_2O_2
15	Chimera	21112333	chimera	89	none	H_2O_2
16	Chimera	21113312	chimera	97	none	H_2O_2
17	Chimera	21113333	chimera	75	none	H_2O_2
18	Chimera	21212233	chimera	89	none	H_2O_2
19	Chimera	21212333	chimera	87	none	H_2O_2
20	Chimera	21311231	chimera	81	none	H_2O_2
21	Chimera	21311233	chimera	97	none	H_2O_2
22	Chimera	21311311	chimera	63	none	H_2O_2
23	Chimera	21311313	chimera	95	none	H_2O_2
24	Chimera	21311331	chimera	81	none	H_2O_2
25	Chimera	21311333	chimera	81	none	H_2O_2
26	Chimera	21312133	chimera	100	none	H_2O_2
27	Chimera	21312211	chimera	76	none	H_2O_2
28	Chimera	21312213	chimera	99	none	H_2O_2
29	Chimera	21312231	chimera	94	none	H_2O_2
30	Chimera	21312233	chimera	96	none	H_2O_2
31	Chimera	21312311	chimera	76	none	H_2O_2
32	Chimera	21312313	chimera	98	none	H_2O_2
33	Chimera	21312331	chimera	94	none	H_2O_2
34	Chimera	21312332	chimera	83	none	H_2O_2
35	Chimera	21312333	chimera	80	none	H_2O_2
36	Chimera	21313111	chimera	58	none	H_2O_2

37	Chimera	21313231	chimera	96	none	H_2O_2
38	Chimera	21313233	chimera	82	none	H_2O_2
39	Chimera	21313311	chimera	78	none	H_2O_2
40	Chimera	21313313	chimera	84	none	H_2O_2
41	Chimera	21313331	chimera	96	none	H_2O_2
42	Chimera	21313333	chimera	66	none	H_2O_2
43	Chimera	21333233	chimera	61	none	H_2O_2
44	Chimera	22112233	chimera	81	none	H_2O_2
45	Chimera	22112333	chimera	93	none	H_2O_2
46	Chimera	22212333	chimera	88	none	H_2O_2
47	Chimera	22223132	chimera	55	none	H_2O_2
48	Chimera	22311233	chimera	92	none	H_2O_2
49	Chimera	22311331	chimera	98	none	H_2O_2
50	Chimera	22311333	chimera	85	none	H_2O_2
51	Chimera	22312231	chimera	78	none	H_2O_2
52	Chimera	22312233	chimera	79	none	H_2O_2
53	Chimera	22312331	chimera	94	none	H_2O_2
54	Chimera	22312333	chimera	84	none	H_2O_2
55	Chimera	22313231	chimera	92	none	H_2O_2
56	Chimera	22313233	chimera	86	none	H_2O_2
57	Chimera	22313331	chimera	102	none	H_2O_2
58	Chimera	22313333	chimera	70	none	H_2O_2
59	Chimera	23132233	chimera	70	none	H_2O_2
60	Chimera	32312231	chimera	101	none	H_2O_2
61	Chimera	32312333	chimera	53	none	H_2O_2
62	Chimera	32313233	chimera	55	none	H_2O_2
63	Chimera	11113311-R1	chimera	35	none	O_2
64	Chimera	12112333-R1	chimera	96	none	O_2
65	Chimera	21113312-R1	chimera	97	none	O_2
66	Chimera	21113312-R2	chimera	97	none	O_2
67	Chimera	21311231-R1	chimera	81	none	O_2
68	Chimera	21311233-R1	chimera	97	none	O_2
69	Chimera	21313311-R1	chimera	78	none	O_2
70	Chimera	21333233-R2	chimera	61	none	O_2
71	Chimera	22132231-R1	chimera	77	none	O_2
72	Chimera	22223132-R1	chimera	55	none	O_2
73	Chimera	22312333-R1	chimera	84	none	O_2
74	Chimera	22313233-R1	chimera	86	none	O_2
75	Chimera	23132233-R1	chimera	70	none	O_2
76	Chimera	23132233-R2	chimera	70	none	O_2
77	Chimera	32312231-R1	chimera	101	none	O_2
78	Chimera	32312333-R1	chimera	53	none	O_2
79	Chimera	32313233-R1	chimera	55	none	O_2

80	Propane	139-3	**WT** V78A H138Y T175I V178I A184V H236Q E252G R255S A290V A295T L353V	10	octane	O_2
81	Propane	J	**139-3** Y138H I178V F205C S226R A290V	10	propane	O_2
82	Propane	9-10A	**J** R47C K94I P142S	13	propane	O_2
83	Propane	9-10A A328F	9-10A A328F	14	propane	O_2
84	Propane	9-10A A328M	9-10A A328M	14	propane	O_2
85	Propane	9-10A A328V	9-10A A328V	14	propane	O_2
86	Propane	9-10A A78F	9-10A A78F	13	propane	O_2
87	Propane	9-10A A78S	9-10A A78S	13	propane	O_2
88	Propane	9-10A A78T	9-10A A78T	13	propane	O_2
89	Propane	9-10A A82C	9-10A A82C	14	propane	O_2
90	Propane	9-10A A82F	9-10A A82F	14	propane	O_2
91	Propane	9-10A A82G	9-10A A82G	14	propane	O_2
92	Propane	9-10A A82I	9-10A A82I	14	propane	O_2
93	Propane	9-10A A82L	9-10A A82L	14	propane	O_2
94	Propane	9-10A A82S	9-10A A82S	14	propane	O_2
95	Propane	9-10A A82T	9-10A A82T	14	propane	O_2
96	Propane	9-10A F87A	9-10A F87A	14	propane	O_2
97	Propane	9-10A F87I	9-10A F87I	14	propane	O_2
98	Propane	9-10A F87L	9-10A F87L	14	propane	O_2
99	Propane	9-10A F87V	9-10A F87V	14	propane	O_2
100	Propane	9-10A L75I	9-10A L75I	14	propane	O_2
101	Propane	9-10A L75W	9-10A L75W	14	propane	O_2
102	Propane	9-10A T260L	9-10A T260L	14	propane	O_2
103	Propane	9-10A T260N	9-10A T260N	14	propane	O_2
104	Propane	9-10A T260S	9-10A T260S	14	propane	O_2
105	Propane	9-10A T88L	9-10A T88L	14	propane	O_2
106	Propane	9-10A A82L A328V	9-10A-A82L-A328V	15	propane	O_2
107	Propane	9-10A A82F A328V	9-10A A82F A328V	15	propane	O_2
108	Propane	9-10A F87V A328L	9-10A F87V A328L	15	propane	O_2
109	Propane	9-10A A82G F87V A328V	9-10A A82G F87V A328V	16	propane	O_2
110	Propane	9-10A A78T A328V	9-10A A78T A328V	14	propane	O_2
111	Propane	9-10A A78T A82G F87V A328L	9-10A A78T A82G F87V A328L	16	propane	O_2
112	Propane	9-10A A82F A328F	9-10A A82F A328F	15	propane	O_2

113	Propane	9-10A A78F A82G A328F	9-10A A78F A82G A328F	14	propane	O_2
114	Propane	9-10A A78F A82S A328L	9-10A A78F A82S A328L	14	propane	O_2
115	Propane	9-10A A78F A82G A328V	9-10A A78F A82G A328V	14	propane	O_2
116	Propane	9-10A A78T A82G F87V A328L	9-10A A78T A82G F87V A328L	16	propane	O_2
117	Propane	9-10A A82G F87L A328L	9-10A A82G F87L A328L	16	propane	O_2
118	Propane	9-10A A78F A82S A328F	9-10A A78F A82S A328F	15	propane	O_2
119	Propane	9-10A A78F A82G A328L	9-10A A78F A82G A328L	15	propane	O_2
120	Propane	9-10A A78T A82G A328L	9-10A A78T A82G A328L	15	propane	O_2

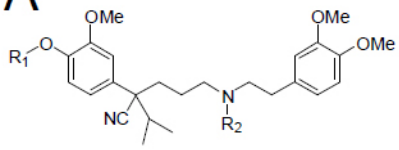
[a]Underlined variants contain wild-type sequences, variants in italics were selected for propranolol activity, variants in bold are chimeras, variants in normal type were selected for activity on alkanes. **21B3** contains the following mutations relative to wild-type: I58V, H100R, F107L, A135S, M145V, N239H, S274T, K434E and V446I. Chimeras are written according to fragment composition: 32313233-R1, for example, represents a protein which inherits the first fragment from parent CYP102A3, the second from CYP102A2, the third from CYP102A3, and so on. The specific amino acid sequence of each block is contained in Table E.2. R1 connotes the presence of the reductase domain from parent A1, indicating that this chimera is a monooxygenase.

Table E.2 Amino acid sequence of blocks 1 – 8 of the cytochrome P450 chimeras

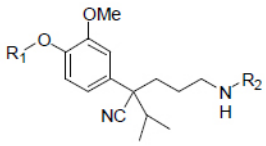
Block	Parent	Sequence
1	A1	-TIKEMPQPKTFGELKNLPLLNTDKPVQALMKIADELGEIFKFEAPGRVTRYLSSQRLIKEACDE
1	A2	KETSPIPQPKTFGPLGNLPLIDKDKPTLSLIKLAEEQGPIFQIHTPAGTTIVVSGHELVKEVCDE
1	A3	KQASAIPQPKTYGPLKNLPHLEKEQLSQSLWRIADELGPIFRFDFPGVSSVFVSGHNLVAEVCDE
2	A1	SRFDKNLSQALKFVRDFAGDGLATSWTHEKNWKKAHNILLPSFSQQAMKGYHAMMVDI
2	A2	ERFDKSIEGALEKVRAFSGDGLATSWTHEPNWRKAHNILMPTFSQRAMKDYHEKMVDI
2	A3	KRFDKNLGKGLQKVREFGGDGLATSWTHEPNWQKAHRILLPSFSQKAMKGYHSMMLDI
3	A1	AVQLVQKWERLNADEHIEVPEDMTRLTLDTIGLCGFNYRFNSFY
3	A2	AVQLIQKWARLNPNEAVDVPGDMTRLTLDTIGLCGFNYRFNSYY
3	A3	ATQLIQKWSRLNPNEEIDVADDMTRLTLDTIGLCGFNYRFNSFY
4	A1	RDQPHPFITSMVRALDEAMNKLQRANPDDPAYDENKRQFQEDIKVMNDLV
4	A2	RETPHPFINSMVRALDEAMHQMQRLDVQDKLMVRTKRQFRYDIQTMFSLV
4	A3	RDSQHPFITSMLRALKEAMNQSKRLGLQDKMMVKTKLQFQKDIEVMNSLV
5	A1	DKIIADRKASGEQ-SDDLLTHMLNGKDPETGEPLDDENIRYQIITFLIAGHET
5	A2	DSIIAERRANGDQDEKDLLARMLNVEDPETGEKLDDENIRFQIITFLIAGHET
5	A3	DRMIAERKANPDENIKDLLSLMLYAKDPVTGETLDDENIRYQIITFLIAGHET
6	A1	TSGLLSFALYFLVKNPHVLQKAAEEAARVLVDPVPSYKQVKQLKYVGMVLNEALRLWPTAA
6	A2	TSGLLSFATYFLLKHPDKLKKAYEEVDRVLTDAAPTYKQVLELTYIRMILNESLRLWPTA
6	A3	TSGLLSFAIYCLLTHPEKLKKAQEEADRVLTDDTPEYKQIQQLKYIRMVLNETLRLYPTA
7	A1	PAFSLYAKEDTVLGGEYPLEKGDELMVLIPQLHRDKTIWGDDVEEFRPERFENPSAIPQHAFKPF GNGQRACIGQQ
7	A2	PAFSLYPKEDTVIGGKFPITTNDRISVLIPQLHRDRDAWGKDAEEFRPERFEHQDQVPHHAYKPF GNGQRACIGMQ
7	A3	PAFSLYAKEDTVLGGEYPISKGQPVTVLIPKLHRDQNAWGPDAEDFRPERFEDPSSIPHHAYKPF GNGQRACIGMQ
8	A1	FALHEATLVLGMMLKHFDFEDHTNYELDIKETLTLKPEGFVVKAKSKKIPLGGIPSPST
8	A2	FALHEATLVLGMILKYFTLIDHENYELDIKQTLTLKPGDFHISVQSRHQEAIHADVQAAE
8	A3	FALQEATMVLGLVLKHFELINHTGYELKIKEALTIKPDDFKITVKPRKTAAINVQRKEQA

Table E.3 Complete list of active enzymes and their metabolite distributions with verapamil

A

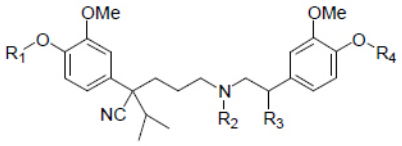


	Mw	R_1	R_2
Verapamil	454	Me	Me
1	426	H	H
2	440	H	Me
3	440	Me	H



	Mw	R_1	R_2
4	276	H	Me
5	276	Me	H
6	290	Me	Me

B



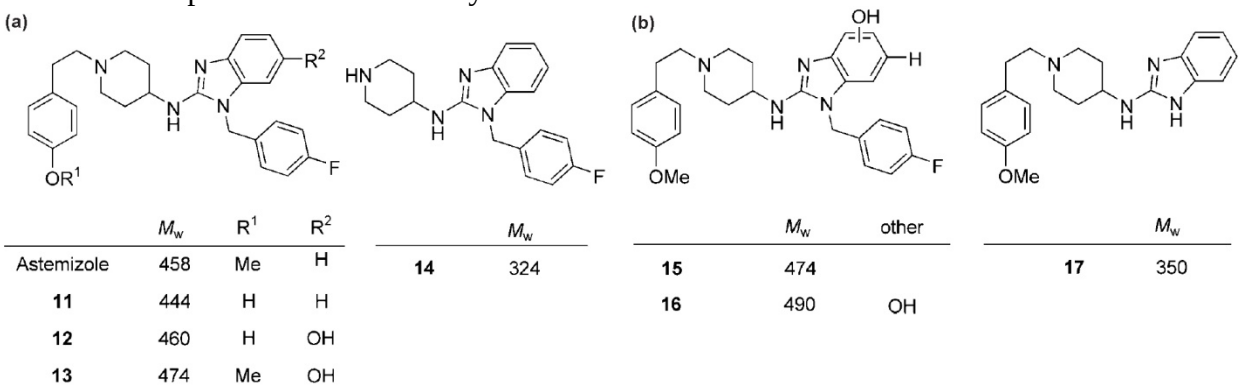
	Mw	R_1	R_2	R_3	R_4	other
7	440	Me	Me	H	H	
8	456	Me	Me	OH	H	
9	470	Me	Me	OH	Me	
10	470	Me	Me	H	Me	OH

Entry	Variant [a]	% Conversion	% 1	% 2	% 3	% 4	% 5	% 6	% 7	% 8	% 9	% 10
1	*2C11*	25		8	28			20	8		8	28
2	*9C1*	31	3		29	3		6	13	3	10	13
3	*D6H10*	78			26	8		31			3	24
4	*DE10*	20						15	35		10	15
5	**11113311**	25			48			28	8			16
6	**21113312**	17		6	12			12	41	6	6	12
7	**21113333**	5						20	40	20		20
8	**21312332**	6			17			33	17			33
9	**21313111**	6						67				33
10	**21313311**	12			33			33	8			25
11	**21313331**	5			20			20	20	20		20
12	**22313231**	43		47				33	5			16
13	**22313233**	12	8					8	17	8	17	8
14	**22313333**	34		41			15	21	9			15
15	**32313233**	24			50			21	13			17
16	**23132233-R1**	15			27			73				
17	**32312231-R1**	23			30			43				26
18	**32312333-R1**	44		7	7				36			
19	**32313233-R1**	25			40			44				16
20	139-3	14		14	14			14	57			
21	9-10A	27		7	7			7	63	7		
22	L75I	34		6	6			6	74	3		
23	A78F	30		10					83			
24	A78S	20			25			35	35			5
25	A78T	25		8	8			8	64	4		
26	A82C	16			6			6	81			
27	A82F	30			10			3	83			
28	A82G	13			31			46	23			
29	A82I	33		3	6			3	79	3	3	
30	A82L	51						2	94			
31	A82S	32		3	3			6	88			
32	A82T	28		4	7			4	86			

Entry	Variant [a]	% Conversion	% 1	% 2	% 3	% 4	% 5	% 6	% 7	% 8	% 9	% 10
33	F87A	12			8			17	58			
34	F87I	13		8				8	69			
35	F87L	49						6	94			
36	F87V	24	4	4				8	63			
37	T88L	14			21			43	21			14
38	T260S	34			12			29	38	9		12
39	A328M	36	3	3	3			6	75		6	
40	1-12G	19			16			21	53			
41	7-11D	21			29			29	14			
42	12-10C	34			6			6	79	3		29
43	41-5B	18			11			6	67			

[a]Variants in italics were selected for propranolol activity (*1*), variants in bold are chimeras (*2*), variants in normal type were selected for activity on alkanes (*3 – 4*).

Table E.4 Complete list of active enzymes and their metabolite distributions with astemizole



	M_w	R^1	R^2
Astemizole	458	Me	H
11	444	H	H
12	460	H	OH
13	474	Me	OH

	M_w
14	324

	M_w	other
15	474	
16	490	OH

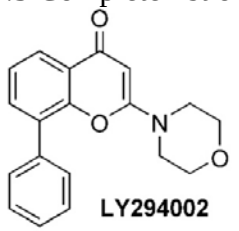
	M_w
17	350

Entry	Variant [a]	% Conversion	% 11	% 12	% 13	% 14	% 15	% 16	% 17
1	*2C11*	4				100			
2	*9C1*	2				100			
3	*DE10*	9				56			
4	**21113312**	6	33			67			
5	**21313111**	4	25			75			
6	**21313311**	10	20			40			40
7	**22313231**	9	22			44			33
8	**22313233**	7	14			57			29
9	**22313333**	9	22			56			22
10	**32312333**	3				100			
11	**32313233**	11				45	27		27
12	**21313111-R1**	11	27		27	18	27		
13	**22313233-R1**	6	33			33			33
14	**32312333-R1**	78				4	67		9
15	**32313233-R1**	16				13	69		
16	139-3	6			67		33		
17	J	23	26	4	65				

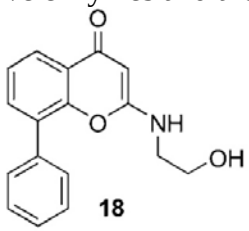
Entry	Variant [a]	% Conversion	% 11	% 12	% 13	% 14	% 15	% 16	% 17
18	9-10A	50	14	10			70	6	
19	L75I	15	33		47				
20	A78F	21	48		38			14	
21	A78S	15	13	7			80		
22	A78T	27	37	7	44			4	
23	A82C	24	33		67				
24	A82F	15	60						
25	A82G	35	29	6	63			3	
26	A82I	32	31	6	63				
27	A82L	49	24				61	4	
28	A82S	30	20	7	70			3	
29	A82T	34	24	12	59			6	
30	F87A	31			23	16	45		
31	F87I	50			48		36	4	
32	F87L	36		3	88			6	
33	F87V	26			38		31		
34	T88L	28			75		25		
35	T260L	2			100				
36	T260N	40		25	75				
37	T260S	25			32	8	44		
38	11-8E	2	100						
39	12-10C	26	38			12	46		
40	23-11B	2	100						
41	41-5B	5	100						
42	49-9B	5	60			40			

[a]Variants in italics were selected for propranolol activity (*1*), variants in bold are chimeras (*2*), variants in normal type were selected for activity on alkanes (*3 – 4*).

Table E.5 Complete list of active enzymes and their metabolite distributions with LY294002



LY294002



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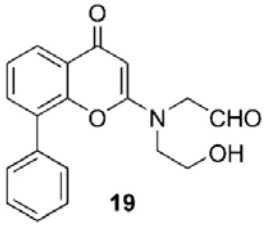

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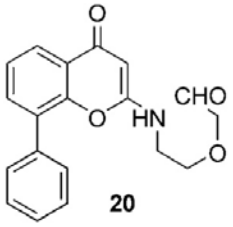

20

M_w: 307 281 323 323

Entry	Variant [a]	% Conversion	% 18	% 19	% 20	% metabolite A [b]
1	**12112333-R1**	8				100
2	**21113312-R1**	8				100
3	**21313111-R1**	7				100
4	**21333233-R1**	4				100
5	**22132231-R1**	7				100
6	**32312231-R1**	6				100
7	**32313233-R1**	8				100
8	J	9				100
9	L75I	10		50		
10	A78S	7		71		
11	A82S	2		100		
12	F87V	6			100	
13	T260S	11		9		91
14	A328F	7				100
15	7-11D	7				100
16	29-10E	12				83
17	68-8F	9		11		89
18	77-9H	11				91

[a] Variants in italics were selected for propranolol activity (*1*), variants in bold are chimeras (*2*), variants in normal type were selected for activity on alkanes (*3 – 4*).
[b] Metabolite of unknown structure, Mw = 238.

1. Otey, C. R., Bandara, G., Lalonde, J., Takahashi, K., and Arnold, F. H. (2006) Preparation of human metabolites of propranolol using laboratory-evolved bacterial cytochromes P450, *Biotechnology and Bioengineering 93*, 494-499.
2. Otey, C. R., Landwehr, M., Endelman, J. B., Hiraga, K., Bloom, J. D., and Arnold, F. H. (2006) Structure-guided recombination creates an artificial family of cytochromes P450, *PLoS. Biol. 4*, 789-798.
3. Meinhold, P., Peters, M. W., Hartwick, A., Hernandez, A. R., and Arnold, F. H. (2006) Engineering cytochrome P450BM3 for terminal alkane hydroxylation, *Advanced Synthesis & Catalysis 348*, 763-772.
4. Peters, M. W., Meinhold, P., Glieder, A., and Arnold, F. H. (2003) Regio- and enantioselective alkane hydroxylation with engineered cytochromes P450 BM-3, *J. Am. Chem. Soc. 125*, 13442-13450.